

THE DEVELOPMENT OF THE PERITHECIA IN THE
MICROTHYRIACEAE AND A COMPARISON
WITH MELIOLA

BY

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THE DEVELOPMENT OF THE PERITHECIA IN THE MICROTHYRIACEAE AND A COM- PARISON WITH *MELIOLA*

RUTH WINIFRED RYAN

(WITH PLATES 12-15)

In recent years many advances have been made in the taxonomy of the fungi. Perhaps in no one group has there occurred as many changes as in the Microthyriaceae. Under the leadership of von Höhnel the entire bases of the family became subject to critical investigation followed by a complete revolution of the classification.

The family was placed among the Dothideales by Saccardo (9) in 1883. Since then it has been described as belonging to the Perisporiales. Atkinson (2), however, considered that they have little in common with this order. He believed that the Microthyriaceae represent reduced forms derived on the one hand from the Phacidiales and, perhaps, on the other from the Sphaeriales. He did not say definitely, though, where they do belong. Theissen and Sydow (15) placed the family in a new order, the Hemisphaeriales, which they created for genera having a superficial, halbert- to shield-shaped peritheciun. Miss Doidge (4) accepts this classification in her systematic work. According to Arnaud (1) the family belongs to the Pyrenomycetes, because, he says, the ascii are localized in particular zones formed by the gelatinization of the sterile cells of the cavity. The Microthyriaceae would thereby be near neighbors of the Myriangiaceae. In my taxonomic study of the Microthyriaceae of Porto Rico (8) and Hawaii (10), I used the classification given by Theissen and Sydow. The Microthyriaceae seem to me more closely related to the Hemisphaeriales than to the other groups with which the family had formerly been placed.

The first description for the family is given by Saccardo (9). Translated, it reads "simple perithecia superficial, black, membranous to carbonaceous, dimidiate, flattened; context nearly

always radiate." This description is accurate. The characters are firmly established and easily recognizable.

The various genera of the family can be placed in two groups according to Theissen (12) and Arnaud (1), those having a free mycelium and those in which it is lacking. The present paper deals with the development of the perithecia in the eleven genera that have a superficial mycelium. The material for study was obtained from the herbarium of the University of Illinois from collections made by Dr. F. L. Stevens in Porto Rico, Hawaii, and British Guiana.

The mycelium in all the eleven genera plays an essential role in the development of the perithecium. Its characteristics are well determined and hereditarily fixed. It is brown, cylindrical, septate, tough and may or may not bear hyphopodia or nodulate cells. These last two structures also play a part in the formation of the perithecium.

Formerly it was thought that the hyphopodia when they occurred on the mycelium of fungi might be sexual organs. This was at first supposed to be the case in *Meliola*. Ward (17) in studying this genus suggested that the hyphopodia were such. More recently Thaxter (11) has compared them to the antheridia of the Laboulbeniales. Arnaud (1) is of the opinion that they do not function as sexual organs and that they are merely the rudiments of the perithecia and absorptive organs. Investigations by Maire (6) show that the hyphopodia in *Meliola* function as organs of absorption and attachment. Cytological studies by Arnaud (1) seem to indicate that the same relation to the host tissue exists in the Microthyriaceae.

Arnaud includes *Meliola* in his study of the Asterinées (1). To me they seem fundamentally different as portrayed in their development. For this reason I have placed them in the Perisporiaceae as does Beeli (3).

The hyphopodia in *Meliola* are two-celled, while they are either one- or two-celled in the Microthyriaceae. In the Microthyriaceae and in *Meliola* they may be opposite or alternate, the majority being the latter. Often one finds several hyphopodia arranged on one side of the mycelium, while further on in the same filament they will be either opposite or alternate. The

characteristic arrangement of the structure may be used as an aid in classification.

The formation of the perithecium in the majority of ascomycetes results from the activity of sexual organs, which have the power of conjugation. This fusion then is the point of development of the perithecium. In *Meliola* (17) the hyphopodia substitute for these organs. Those that give rise to perithecia have a reflexed swollen end and produce the fruiting structures through proliferation. In the Microthyriaceae the hyphopodia though not sexual organs give rise to the perithecia in certain cases. This family advances further, for here the perithecia may arise independently of the hyphopodia, that is, from the mycelial cells themselves. This method of origin seems to be by far the most common.

A search through mycological literature revealed only a few articles which bear in any way on the development of these fungi. Gaillard (5) studied a few species of *Asterina*. He says that the summit of the hyphopodium turns toward the leaf. This small cell divides, at the same time becoming clearer. The primitive membrane becomes thinner, budding in places and appearing as a small lenticular body closely applied against the leaf. Its surface is covered by deep lobes, which in growing seem to radiate around a point, which is the place of insertion on the mycelium of the primitive hyphopodium. In the majority of cases the hyphopodia do not participate alone in the division, but the portion of the mycelium where it is inserted also takes an active part. Thus he indicates one method of development characteristic of the genus and of the family as a whole.

Raciborski (7) refers to the subject only in passing, saying that the perithecia are borne laterally on the hyphae as in *Balladyna*, *Aldona*, *Dimerosporium*, or *Meliola*, and that they grow conical and broaden against the leaf. Miss Doidge (4) briefly gives two methods; from a medial cell of a hypha and from the terminal cell of a short lateral branch. She does not go into any detail.

Theissen (13) says that divisions in the hyphae give rise to irregular cells by the insertion of several cross walls. The perithecium arises on the lower surface of the hyphae. A small

knot of cells is formed and is the base of the perithecium, which continues to grow radially and centrifugally to a disk-shaped structure. The radial walls remain locked together. New radial walls become inserted so that the disk shape is maintained.

Arnaud (1) thinks that the fertile stroma arises by the proliferation of a cell or a small number of cells which put out filaments extending radially on the cuticle of the host, and which fork enough to form a continuous bed. These proliferations constitute a regular radiate disk. As this plate becomes thickened on its inferior face, the superior face conserves on all its extent the radiate structure.

Von Höhnel (16) states that the shield-shaped structure is merely a protective cover for the independent perithecia which develop inversely beneath it.

Raciborski (7) was the first, however, to state that the perithecia were inverse. Theissen, Miss Doidge, Arnaud, and von Höhnel have accepted his statement as being correct. By inverse, one understands the perithecia to be fastened on the under side of the hypha. The oldest or basal part therefore is above the newest or marginal part of the fruiting body. Von Höhnel also pointed out that the asci arise from the part of the perithecium pressed against the leaf, or from the youngest and the top part of the structure. The asci are, therefore, inverted in the perithecium, but are borne upright on the surface of the leaf. This meaning of the term inverse has been used in this discussion.

As I have stated previously, in *Meliola* the fructifications are always derived from hyphopodia, while in the Microthyriaceae in certain instances the perithecia are formed from the hyphopodium; in other instances from the cells of the mycelium. This latter method is characteristic of species which have a non-hyphopodiate mycelium, but which form as many fructifications as the others.

The origin directly from the mycelium predominates throughout all the genera of the family. Out of the ninety-four species studied eighty-four show this method of development.

Portions of the mycelium were fixed in celloidin, mounted and studied under oil immersion. The mycelium sometimes was slightly swollen in regions where a perithecium was developing.

At other times it remained normal. In either case, however, cross septa appeared, giving rise to one or more small cells. Rarely does the number exceed five. These cells are nearly square in shape, otherwise showing no differentiation from the rest of the mycelium. They all seem to function alike in the formation of the perithecium (PLATE 12, FIG. 8).

They give rise to bud-like growths (PLATE 12, FIG. 4). These budded cells are irregular in outline, often distinctly lobed on their free margins. They may occur on both lateral margins of the mycelium. The budded cells likewise divide increasing the number of cells centrifugally. These cells are united to one another so that a disk-like structure is produced (PLATE 12, FIG. 6). Further cells are added to the structure until finally the maximum growth of the perithecium is reached. The walls of these cells are thick and dark. There may be more than one layer of them. Cross sections of the perithecium generally show at least two.

The second method of origin is from hyphopodia which are one- to two-celled structures located on the lateral margins of the mycelium. If the hyphopodium has only one cell, this cell buds several times (PLATE 14, FIG. 43). The budded cells in turn proliferate, the new cells being laid down in rows (PLATE 14, FIG. 43). This process continues until the maximum growth has been reached. The radiate structure can be observed after several rows of cells have been formed.

If the hyphopodium is a two-celled structure, the terminal cell only participates in the formation of the perithecium. This cell buds in the same manner as does the one-celled hyphopodium. In both kinds of hyphopodial development, as well as in the development from the mycelium, the perithecium produced is always inverse at maturity.

A third type of development is from short lateral branches. This method is less common; only six species of all those investigated show this method of origin. The lateral branch of the mycelium, consisting of one to three cells, is ordinarily borne at an acute angle (PLATE 12, FIG. 14). The terminal cell in all cases is the one that functions in the production of the fruiting structure. This cell buds in a manner like that of the terminal

cell of the hyphopodium. *Asterina Gouldiae* is a typical example of this mode.

Only two species having nodulate cells in the mycelium were studied. These were *Asterina inaequalis* var. *nodulosa* (PLATE 12, FIG. 13) and *Asterina Schroeteri* (PLATE 13, FIG. 15). In both these forms the peritheciun did not develop from the nodulate cell, but from an undifferentiated cell of the mycelium. The manner of growth was like that already described in the first method.

Gaillard (13) gives illustrations of *Asterina stricta* which has nodulate cells that develop into the perithecia. This would indicate that there were two ways of producing a peritheciun in the species which possess this kind of a mycelium. A further study of other species with a like mycelium would be interesting and would enable one to form a more accurate idea of the true method of origin in these species.

In all the genera after the disk has reached its specific extension the radial growth is halted. The central part has meanwhile swollen from the middle of the perithecial hollow, while the peripheral growth remains close to the leaf. The result is a concave peritheciun, which is only half a one in reality and is developed below the mycelium.

The preceding description of the development of the peritheciun can be applied to all the genera, for even those having oblong perithecia at maturity are at first circular. The elongate character is due to the more rapid growth of the peritheciun on two opposite points on the disk. Frequently a three-angled peritheciun is observed. This seems to be the result of growth at three points on the disk instead of at two.

Contrasted to these various methods of origin, the perithecia in *Meliola* exhibit an unexpected constancy. In the forty species studied, all showed origin from the hyphopodia. Furthermore, the method of development observed agreed in detail with that reported by Marshall Ward (17). A brief summary is given here.

The simple pyriform body, the hyphopodium, after becoming swollen, suffers division into two portions or cells by a septum, usually vertically to the plane of the mycelium and leaf, and passing diagonally across the cavity with a slight curve so as to

abut on the outer walls at right angles, or nearly so. The original unicellular protuberance becomes in this manner divided into two more or less unequal cells which have different destinies. The more apical cell divides more slowly and forms a mass of cells, the central ascogenous tissue, of the young perithecium. The outer cell divides much more rapidly, producing a layer of cells which gradually develops the thin-walled cells of the outer portion of the perithecium. These thin-walled cells make the ascogonium. In later stages, according to Ward, certain of the constituents are seen to form asci and spores, while others deliquesce and serve as nutritive material. The outer walls, as in the Microthyriaceae, become thick, hard, and dark colored. The perithecium in *Meliola* is always produced from the upper side of the mycelium so that it is never inverse (PLATE 15, FIG. 1) and is always spherical or approximately so.

While this method of development holds for the majority of the species of *Meliola*, there are six species which show more distinct relationship to the Microthyriaceae. Beeli in his monograph of the genus *Meliola* (3) makes a division on the character of the radiate and pseudoparenchymatous tissue, placing the six species referred to in one group. He seems to have either overlooked the previous work of Theissen on *Meliola* (14) or to have rejected it.

Theissen studied specimens of *Meliola asterinoides* and created for it a new genus, *Amazonia*, which he placed in the Microthyriaceae because of its radiate inverse perithecia. In studying material labeled *Meliola Psychotriae*, one of the other five species in this group, I have found that the development of the fruiting body approximates the development of the perithecium in the Microthyriaceae. The origin is from the hyphopodium, which proliferates in the manner already described for the hyphopodial origin in the Microthyriaceae. There is no definitely arranged method of division, as in *Meliola*, for the cells formed may or may not be of the same size. Furthermore, there may be more than two cells produced. In any circumstance the cells do not seem to form definite regions of the perithecium as in *Meliola* (PLATE 12, FIG. 7). At maturity or during the latter part of growth, the fruiting structure is inverse and radiate, thus differing

in two essential characters from *Meliola*. For these reasons I have followed Theissen's classification and placed the fungus in the genus *Amazonia*.

Meliola Lagunculariae, like the foregoing species, is also inverse radiate (PLATE 15, FIG. 15). The method of development of the perithecium is like that described above. Therefore, this species should also be placed in the genus *Amazonia* as *Amazonia Lagunculariae* comb. nov.

I did not study the other species listed by Beeli in his first division, but in all probability they will show the same characters as the two foregoing species and, therefore, will be placed in the same genus.

In some of the Microthyriaceae an ostiole-like opening appears. This is not an "ostiole" in the true sense of the word. It appears at maturity and is due to the gelatinization or cracking of the central cells around the point of insertion on the mycelium or hyphopodium. The region where this occurs is the oldest and basal portion of the perithecium, and not the youngest and top part as one would ordinarily expect. There is evidently no precise method of development for the "ostiole." When the perithecium is round the opening may be circular with ragged edges, due to the loss of several of the central cells (PLATE 14, FIG. 40), or it may be stellar, due to the severance of some of the cells along the lateral walls (PLATE 14, FIG. 33). Frequently these fissures extend almost to the margin of the perithecium. Or the entire central region of the perithecium may become gelatinous and disappear, leaving the asci fully exposed.

In genera where the perithecia are oblong in shape, the "ostiole" follows the shape of the fruiting structure. It may remain narrow, so that it is thread-like in appearance, or it may become greatly widened, in which case the asci are exposed. In these oblong perithecia the lenticular "ostiole" may become stellar at its ends.

"Ostioles," however, do not occur in all the species before they reach maturity. Before the dispersal of the spores can take place though, the shield-like structure must be ruptured or become disintegrated. In *Meliola*, the rupturing of the perithecium occurs in the youngest and the top part of the peri-

thecium. This is diametrically opposed to that of the Microthyriaceae. In *Amazonia Psychotriae* the "ostiole" is like that of the family to which it belongs.

The typical young fruiting body in the Microthyriaceae is always light brown in color. The radiate cell structure is apparent. On maturity it often becomes a dark brown, darker than the mycelium. The margin, though, remains lighter in color and the radiate character persists. At times, though, the radiate structure is not apparent, until the material has been boiled in potassium hydroxide, or a young specimen has been observed. When the first cells of the perithecium are laid, they ordinarily are arranged in a row. The next cells also occur in a row, and it is not until then that the perithecium begins to look radiate. On maturity, too, this character may be nearly obscured. In some genera the central cells gelatinize, so that only the marginal cells remain intact. These, however, always show the character. Other genera have perithecia which become almost wholly carbonaceous. But in such cases the margin again gives the character.

SUMMARY

The Microthyriaceae show four characteristic ways of developing the perithecia. The methods are: from a cell of the mycelium; from hyphopodia; from the short lateral branch; from a nodulate cell. The most common way is from a cell of the mycelium.

The perithecia are flattened.

In early stages the fruiting structure is radiate. This character later may be lost due to the perithecia losing its top through becoming gelatinous or carbonaceous. However, at all times the edge of the structure will show the character.

The perithecia are all inverse, that is, they are borne on the lower surface of the mycelium.

At maturity the perithecia break open, thus permitting the spores to escape. Often, however, an "ostiolar" opening appears at the base of the perithecium. This may be round, stellar, or linear in shape.

In *Meliola* the perithecia always arise from hyphopodia.

The perithecia are more or less round, never flattened. They never show the radiate character at maturity, although a few species may do so when very young.

The perithecia are never inverse, but are always borne on the upper surface of the hyphopodium.

Meliola Psychotriae is radiate and inverse as is also *Meliola Lagunculariae*; they, therefore, belong to the genus *Amazonia*.

An undetermined perisporiaceous form also showed the radiate perithecia and so rightfully belongs to Microthyriaceae.

The following table lists the genera of the Microthyriaceae examined in this study. Hypho. stands for hyphopodia; myc., mycelium; lat. br., lateral branch; nod., nodulate cell.

Genus	Method of Development				Ostiole		
	Myc.	Hypho.	Lat. Br.	Nod.	Linear	Stellar	Round
<i>Asterina</i>	35	3	2	2	7	7	11
<i>Asterinella</i>	6	1	1			4	7
<i>Amazonia</i>		1	1				1
<i>Aulographum</i>	1				1	1	
<i>Calothyriopeltis</i>	3					1	
<i>Echidnodes</i>	3				3		
<i>Echidnodella</i>	5				5		
<i>Englerulaster</i>	2		1				3
<i>Lembosia</i>	10				10		
<i>Morenoella</i>	16				16		
<i>Questieria</i>	1		1				3
Method of Development							
			Hypho.		Inv.	Not Inv.	Rad.
<i>Meliola</i>			40		2	38	4
							36

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EXPLANATION OF PLATES

All figures were drawn from material mounted in celloidin and drawn with the aid of a Spencer camera lucida, and were reduced one half in reproduction. PLATES 12 to 15 show stages in the development of the perithecia in the various species studied.

PLATE 12

Fig. 1, *Morenoella decalvans* var. *Stigmatophyllii*; 2, *M. Calami*; 3, *Asterinella multilobata*; 4, *A. Hippaeastri*; 5, *A. Ixorae*; 6, *A. Phoradendri*; 7, *Amazonia Psychotriae*; 8, *Calothyriopeltis Scaevolae*; 9, *Lembosia Sclerolobii*; 10, *L. Coccolobae*; 11, *L. ananensis*; 12, *L. portoricensis*; 13, *Asterina inaequalis* var. *nudulosa*; 14, *A. Gouldiae*.

PLATE 13

Fig. 15, *Asterina Schroeteri*; 16, *A. acanthopoda*; 17, *Morenoella decalvans* var. *Rondeletiae*; 18, *M. Pothoidei* var. *Laevigatae*; 19, *M. Psychotriae*; 20, *M. decalvans* var. *Laugeriae*; 21, *M. Laugeriae*; 22, *M. miconicola*; 23, *M. impetolaris*; 24, *M. Miconiae*; 25, *M. Melastomacearum*; 26, *Asterina diplocarpa*; 27, *A. Arnaudia*; 28, *A. Chrysophylli*; 29, *A. dilabens*; 30, *A. Rickii*; 31, *A. Clermontiae*; 32, *A. Lobeliae*; 35, *A. dubii*.

PLATE 14

Fig. 33, *Asterina Ildefonsiae*; 34, *A. Fawcetti*; 36, *A. Hippocrateae*; 37, *A. correacola*; 38, *A. chrysophylliella*; 39, *A. kauaiensis*; 40, *Englerulaster orbicularis*; 41, *E. Papawiae*; 42, *Aulographum culmigenum*; 43, Unidentified Perisporiaceae.

PLATE 15

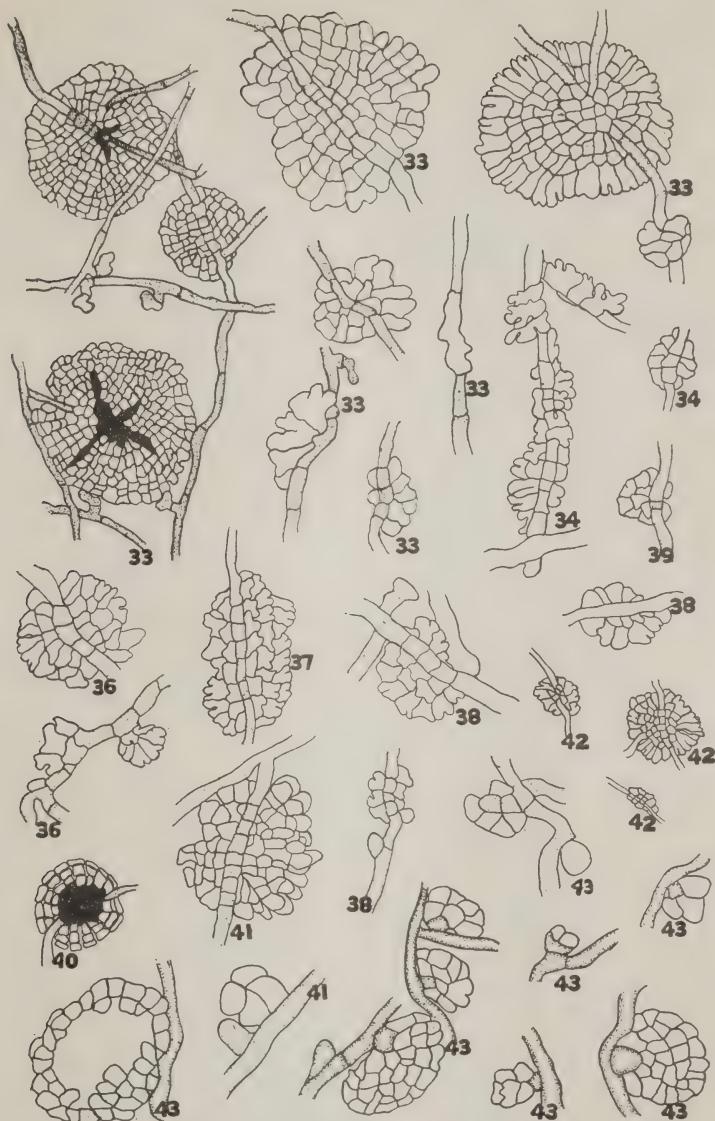
Fig. 1, *Meliola irregularis*; 2, *M. Lyoni*; 3, *M. Kaduae*; 4, *M. Koae*; 5, *M. maricaensis*; 6, *M. bidentata*; 7, *M. longipoda*; 8, *M. brasiliensis*; 9, *M. Dieffenbachiae*; 10, *M. Calophylli*; 11, *M. Juddiana*; 12, *M. hyptidicola*; 13, *M. Cyperi*; 14, *M. Kaduae*; 15, *M. Lagunculariae*; 16, *M. cyclopoda*; 17, *M. Marantae*; 18, *M. toruloidea*; 19, *M. brasiliensis*.



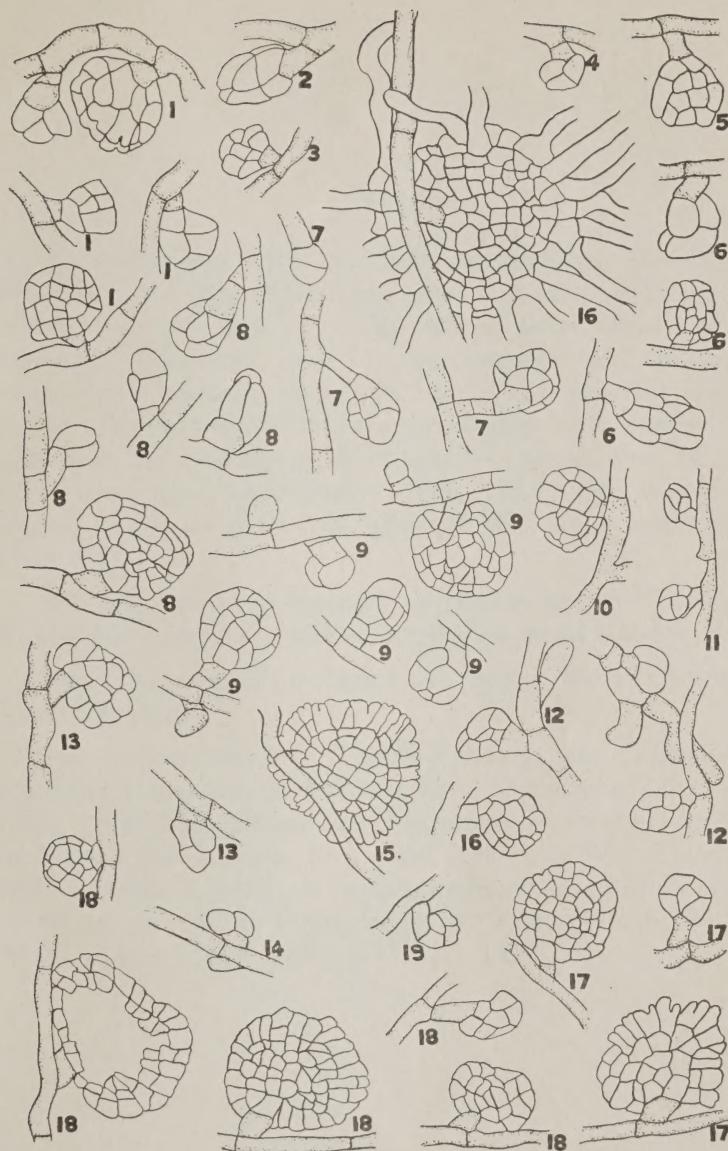
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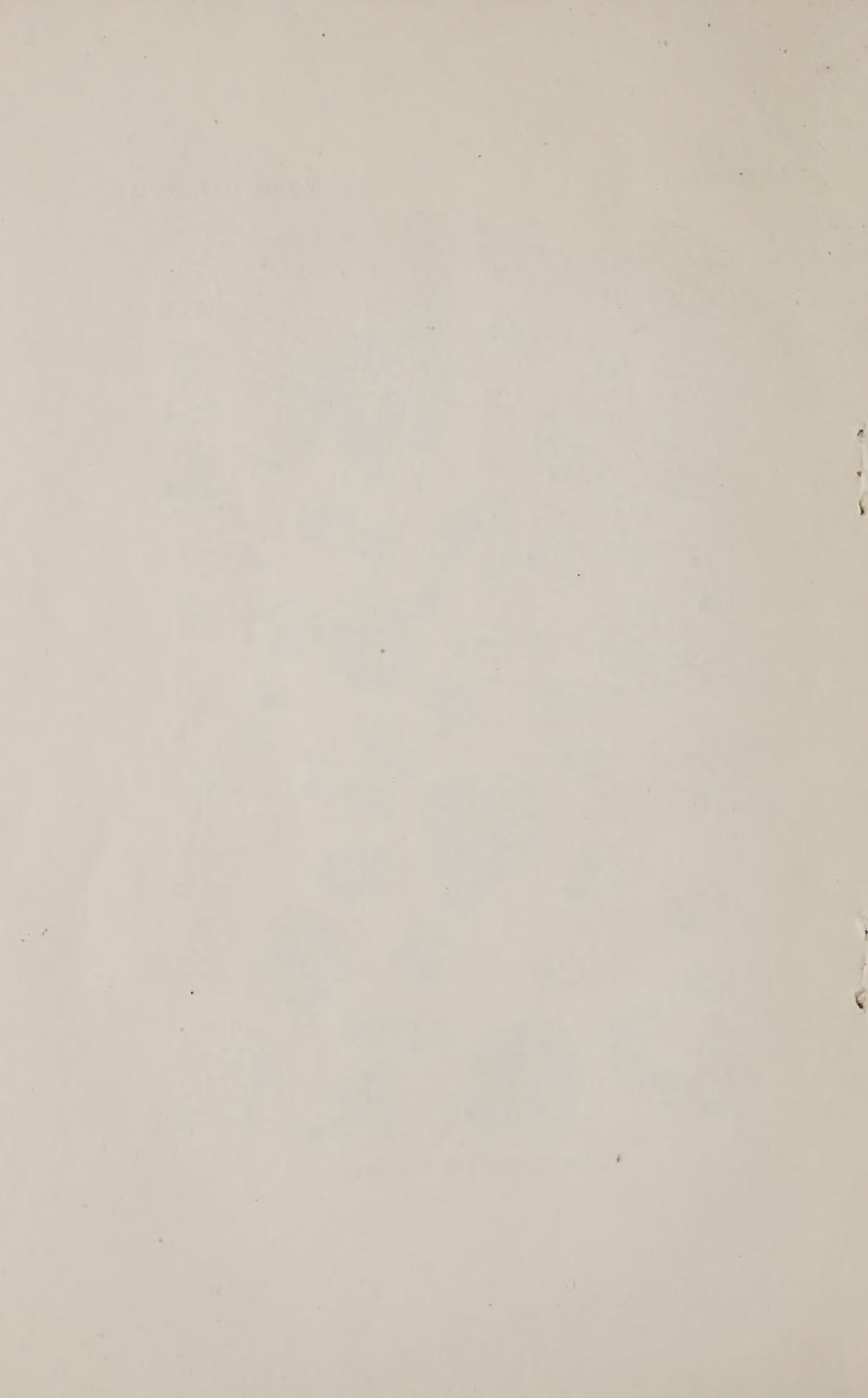
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DEVELOPMENT OF PERITHECIA



VITA

Ruth Winifred Ryan was born at Appleton, Wisconsin, on June 19, 1899. She received her grade and high school education in the schools of that city. She entered Saint Clara College at Sinsinawa, Wisconsin, in 1917, and completed her undergraduate work with a major in botany in June, 1921, receiving the A. B. degree. She entered the University of Illinois in September, 1921, and began her graduate work in botany. She received the A. M. degree from this institution in June, 1923. She was graduate student in botany at the University of Wisconsin during the year 1924-25.

She was half-time Assistant in Botany at the University of Illinois for three years, where she taught general botany.

She is a member of Sigma Xi, Sigma Delta Epsilon, and Theta Phi Alpha.

Her publications are: The Morphology and Taxonomy of the Microthyriaceae of Porto Rico. *Mycologia*, 16:177-196. 1924. The Morphology and Taxonomy of the Microthyriaceae of Hawaii, by Stevens, F. L. and Ryan, R. W. Bulletin 10, Bishop Museum, 1925. A Systematic Presentation of New Genera of Fungi, by O. A. Plunkett, P. A. Young, Ruth W. Ryan. *Trans. Amer. Micro. Soc.* 32:43-67. 1923.



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